SOME OBSERVATIONS ON THE GOLGI MATERIAL IN THE LARVAL EPIDERMAL CELLS OF DROSOPHILA MELANOGASTER

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Introduction

In my attempt to make a general survey on the morphology and behavior of Golgi material in a number of tissues of the larvae of Drosophila melanogaster, I have encountered in the larval epidermal cells some rather interesting phenomena which form the substance of this paper. These phenomena, besides confirming the facts and conclusions reported in my previous papers dealing with Golgi material in the larval tissues of the same fly (1947, 1948), seem to point rather definitely to a hitherto unreported function of the epidermal cells of Drosophila larvae—that of internal secretion.

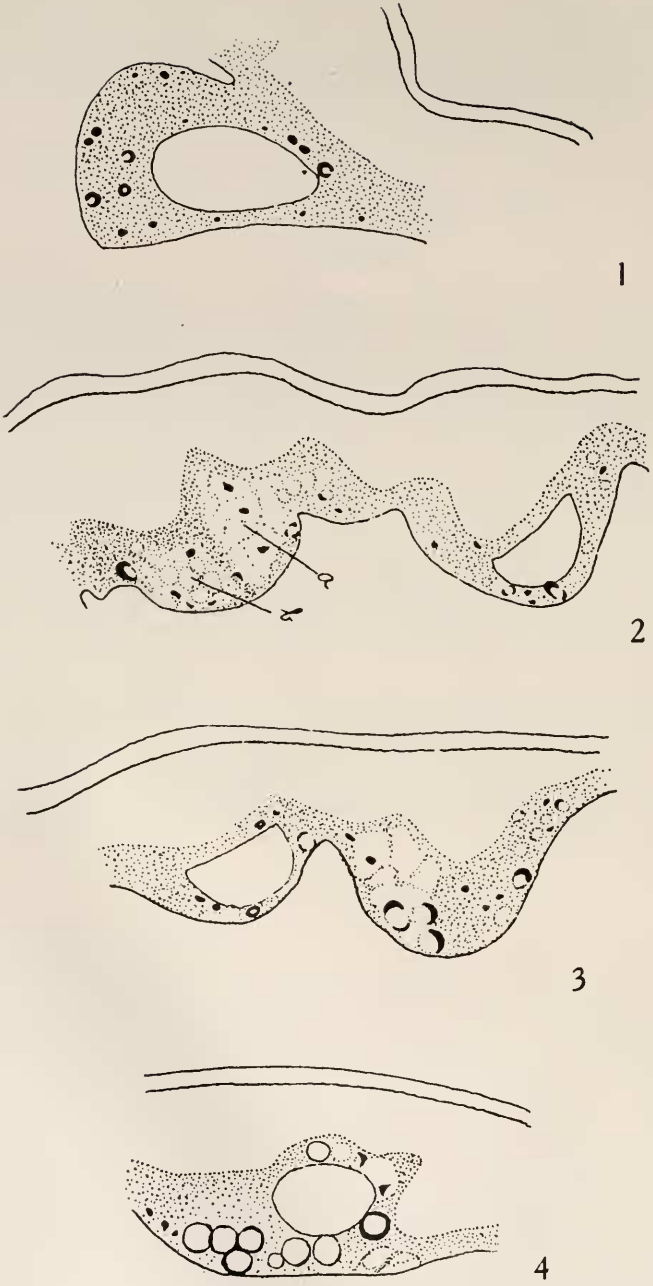
The larvae used for this study were from "wild flies." They were raised in the same cultural conditions as those used for the mid-gut study (1947). The undesirable effect of crowding the larvae was avoided by placing four or five females for only 24 hours in each bottle of 100 c.c. capacity containing approximately 10 cc. of food. Besides, only those which first reached the desired age in a fresh bottle were fixed.

For Golgi material observations, Koletchev and Mann-Kopsch techniques were employed; but the Golgi bodies in the epidermal cells, as in practically all other tissues studied in the larvae of this fly, seem to show up far better in the Mann-Kopsch slides. These slides were so much relied upon for critical observations that all figures except Figure 5 in this paper were drawn from them.

Observations and Discussion

In a section taken almost at any point along the length of a larva, most of the epidermal cells would present such appearances as shown in Figures 2 at a, 7, and 8. The cytoplasm in these cells is extremely vacuolated; and, in the vacuolated areas, apparently homogeneous bits of Golgi material of various shapes are observable. Careful focussing on these vacuolated areas, however, would usually show that they are the results of a confluence of individual small spherical globules. Evidences supporting such a view are easily obtained, once the observer is made aware of the situation. The apparent unity of many of these big and irregularly-shaped vacuolated areas would often resolve, upon careful study, into many individual small spherical globules, each still surrounded by a very thin layer of cytoplasm (Figs. 2 at b, 5 at a, 6). So, when a large irregularly-shaped vacuole is seen in a cell, it may be in a stage wherein its contributing small globules have all lost their individuality, the thin layer of cytoplasm having been withdrawn from around them (Figs. 7, 8). However, it may also be in a stage when the individual small compo-

PLATE I



nent globules can still be made out unmistakably (Figs. 2 at b, 5, 6). The smaller globules found within a large vacuolated area are of various sizes; and it should be noted that their sizes correspond very closely to those of the globules observed elsewhere in the cytoplasm where the formation of a large irregularly-shaped vacuole cannot as yet be easily suspected (Figs. 2, 3, 6). This is pointed out as an evidence, aside from what is indicated by the relation between the globules and the Golgi material, which will be discussed presently, that the globules both within and without the large vacuolated areas are of the same category of entities, and therefore they should have a similar origin.

Upon examining a cell at a stage of vacuolation as shown in Figures 2 at a and 7, it would be difficult to see any relation between the bits of Golgi material and the big vacuoles. But the condition as illustrated in Figure 6 reminds me rather strongly of the behavior of Golgi material in cells actively engaged in synthesizing secretion, such as have been observed in the cells of the mid-gut epithelium (1947) and those of the salivary glands of a Drosophila larva (1948). The appearance of the Golgi material in its relation to the spherical globules depicted in the same figure recalls its condition seen in the cells of both the tissues mentioned above when the individual secretion granules or droplets have grown to such a size as to be ready to be freed from the confining Golgi shell. The granular appearance of the Golgi rim around the globules is characteristic of the Golgi material when its separation product is ready to be released and itself about to break into bits of irregular shape, each of which is presumably capable of starting another cycle of secretion when proper conditions again prevail.

In addition to the observations mentioned above, my experience with the behavior of Golgi material in the glandular cells of Drosophila larvae inclined me to believe that two or three pieces of Golgi material seen in the cell represented by Figure 2 also showed familiar signs of being in the act of separating some kind of secretional material in their interior. These observations led me to a search in a large number of slides for more definite evidence in that direction. My effort was rewarded with a number of cells such as represented by Figures 3 and 4. In these

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Explanation of Plates

All the figures of the two plates are camera lucida drawings at a magnification of approximately 2200 ×.

Golgi material is represented as black dots, crescents or rings; secretion globules, as circles of broken lines; and cytoplasm, as stippled areas. A nucleus, whenever included in a figure, is indicated by an unstippled area marked out with a solid line. The epidermal cells are depicted in the figures as being over-laid with two layers of cuticula.

Plate I

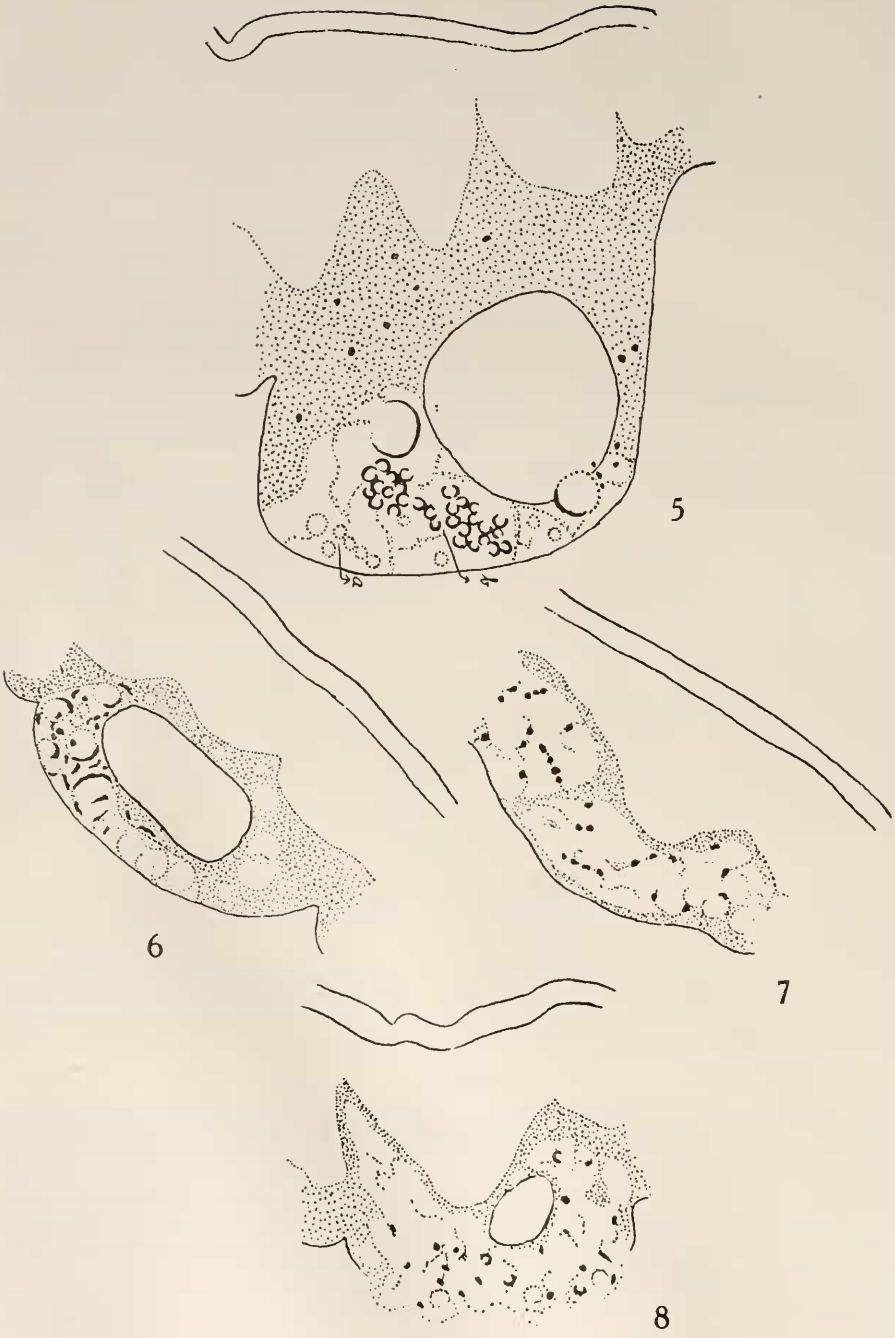
Figure 1. A cell showing in the cytoplasm some pieces of Golgi material which are apparently homogeneous and some in each of which a light center has become visible; no free secretory globules are yet observable.

Figure 2. Two cells: the one to the right showing a number of free secretory globules in the cytoplasm; the one to the left showing at b a few free globules about to be fused to produce in the cytoplasm a condition such as depicted at a.

Figure 3. Two cells showing practically all the stages of the development of a secretion globule in relation to Golgi material.

Figure 4. A cell showing the unmistakable origin of the secretory globules in the Golgi material.

PLATE II



two cells, a Golgi-material origin of the spherical globules is unmistakable. Figure 3 shows practically all the stages of the development of a secretion globule in relation to Golgi material, from apparently homogeneous bits of Golgi material to mature globules lying free in the cytoplasm.

Thus, regarding the origin, growth and releasing of the secretion globules in relation to Golgi bodies, the situation as found in the epidermal cells may be summed up as follows: Inactive pieces of Golgi material appear homogeneous; but when secretory synthesis has proceeded sufficiently far, there becomes visible one light area in each Golgi body. This area has been interpreted, on evidences which have been reported in a previous paper (1947), to be a globule of elaboration product viewed through a layer of Golgi material. As the globule increases in size, the light area in most of the Golgi bodies becomes less and less colored as a result of the continual thinning of the overlying layer of Golgi material on the surface of the globule. A time will eventually be reached when the Golgi shell will no longer be able to contain the enlarging globule within it and will mechanically break into small irregular pieces, releasing its contents into the cytoplasm. This series of changes almost exactly duplicates what has been seen in the other larval tissues of Drosophila definitely known to have a glandular function (1947, 1948).

What I consider as most instructive and significant, however, are strips of cells, usually five to six in number, which have often been observed with their cell membrane broken but with their nuclei and part of their cytoplasm still intact (Fig. 8). These cells remind one most vividly of the method of discharging their secretory product seen in the larval mid-gut epithelium and salivary gland cells of this same fly (1947, 1948). When one adds to this situation the relation observed to be existing between the Golgi bodies and the globules which they elaborate, as pointed out in the preceding paragraph, the suggestion becomes more than probable that in the Drosophila larvae, the epidermal cells may also serve as internal secretion glands. Thus, Figures 1 to 7 may be taken as showing graphically the various stages of secretory synthesis which an epidermal cell passes through—from a stage wherein the cytoplasm contains no free secretory globules but numerous Golgi bodies (some are visibly homogeneous while others show a light center) to a stage in which the cell is extremely vacuolated due to the confluence of a large number of secretory globules elaborated and set free in the cytoplasm by the Golgi bodies. Figure 8, finally, illustrates the merocrine method of discharging secretion by the cell. Having failed to see any replacement cells in the epidermis, I assume that after having

PLATE II

FIGURE 5. A cell showing the vacuolated condition of the cytoplasm near the basement membrane. A few unfused globules are still visible within the vacuolated area at a; a group of Golgi bodies all at about the same stage of secretory synthesis are seen at b. Note the two large globules not yet freed from their respective Golgi shell and also the bits of apparently homogeneous Golgi material in the cytoplasm near the cuticula end of the cell.

FIGURE 6. A cell showing the Golgi shells around the globules already or about to be broken into small pieces which have the rugged surface and irregular shapes characteristic of apparently homogeneous and inactive bits of Golgi material.

FIGURE 7. A cell showing its cytoplasm extremely vacuolated with apparently homogeneous bits of Golgi material embedded on strands of cytoplasm; at the right end of the cell may yet be seen some globules in the process of fusing.

FIGURE 8. A cell with its cell membrane broken discharging its secretion and also a portion of its Golgi material into the body cavity of the larva.

discharged its store of secretion, each cell is capable of repairing itself and starting another cycle of secretion when proper conditions are again present. This is the situation which prevails in both the mid-gut epithelium and the salivary gland cells.

It is interesting to record that it was after I had reached the conclusion that the cells of Drosophila larvae may serve as internal secretion glands that my search into the literature for some supporting opinion led me to a paragraph by Wigglesworth (1934) in his study on ecdysis in Rhodnius, which I quote: "The histological evidence therefore favours the idea that the corpus allatum is responsible for secreting the moulting hormones which must be derived from the growing cells themselves, and this raises the question whether the general epidermal cells may not be responsible for the initial moulting hormone. This possibility cannot be entirely excluded; but the epidermal cells are not innervated, and it is therefore probable that any hormones they secrete appear only when their own growth has been stimulated by the hormone from the head." This is the first reference to the epidermal cells as internal secretion organs which has come to my knowledge, although it must be admitted that my search in the literature in that regard is not an exhaustive one. However, in quoting Wigglesworth, I do not claim that my observations prove that the epidermal cells are "responsible for the initial moulting hormone" in Drosophila larvae. Needless to say, this question merits more particular examination. I only claim that according to my material it is difficult to dismiss the idea that the epidermal cells of a Drosophila larva are capable of secreting some substance and that the secretion is discharged directly into the body cavity of the larva.

Summary

1. On the strength of observations set forth in the following paragraphs, it has been concluded that the epidermal cells of Drosophila larvae seem to act as internal secretion organs, at least at the age when the larvae are about one day before pupation.

2. The relation of the Golgi bodies to the globules, both inside and outside of the Golgi bodies as observed in the epidermal cells, has been found to be the same as what has been established in cells definitely known to be of a glandular nature in the larvae of this fly. In each piece of Golgi material, a single droplet is seen to make its first appearance and gradually to increase in size, eventually breaking free from the confining Golgi shell. It seems to be the normal procedure for the free separate secretory droplets to coalesce to form big vacuoles; and their further confluence gives to the cells in advanced secretory synthesis an extremely vacuolated appearance.

3. Many epidermal cells have been found with their cell membrane broken, thus releasing their secretion product into the body cavity of the larva. The apparent healthy condition of the nuclei of such cells and the absence of replacement cells in the epidermis would point to a merocrine mechanism of secretion in this case.

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